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GRAND ROUNDS

Parkland Memorial Hospital February 1, 1962

"CONNECTIVE TISSUE METABOLISM IN NORMAL GROWTH, ACROMEGALY AND DWARFISM"

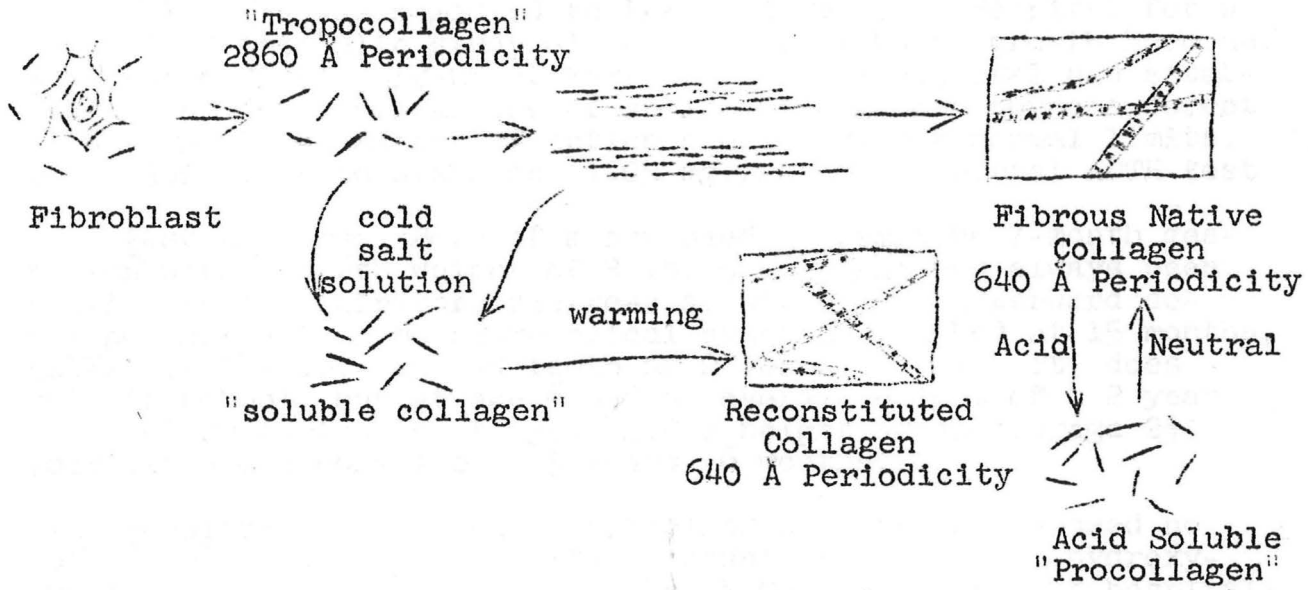
The diseases of connective tissue include the so-called "collagen diseases" which encompass some of the most disabling diseases of man, as well as several rare inherited disorders and even the growth and aging process itself. The metabolic bases of these diseases are not understood, and for this reason their study, as well as the study of normal connective tissue, has attracted increasing interest in the past few years (1). Greatest progress has been made in analytical and morphological areas, and much has been learned about the characteristic features and variations of the body's largest single organ. Collagen, which comprises 30 per cent of the total body protein, has been well defined by excellent electron microscopic (2) and chemical studies (3). The several acid mucopolysaccharides which contribute significantly to the extracellular ground substances have been isolated and thoroughly analyzed (4). Elaboration of both collagen and mucopolysaccharides has been demonstrated in tissue cultures of fibroblasts (5) and within polyvinyl sponge implants (6). Various biosynthetic pathways of the simple sugar and amino acid building blocks of connective tissues and their nucleotide-linked forms (7) have been almost completely worked out. And finally, several heritable disorders of connective tissue have been critically re-examined (8).

A brief review of the most recent information concerning the composition of the cellular, fibrous and ground substance elements of connective tissue and their normal metabolism will be presented. An analysis of the changes induced in this metabolism by normal growth and in the disease states of abnormal growth, acromegaly and dwarfism, will be discussed. Special emphasis will be given to those diseases in which collagen metabolism produces an elevation of the urinary hydroxyproline peptide excretion.

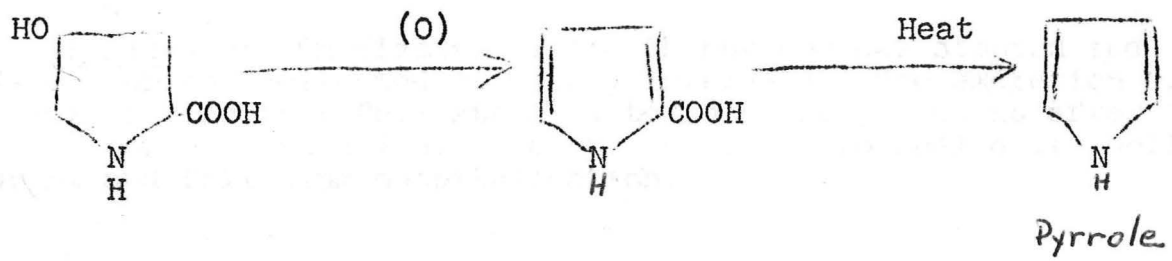
THE ACID MUCOPOLYSACCHARIDES OF CONNECTIVE TISSUES AND THEIR PROPERTIES

TRIVIAL NAME	HYALURONIC ACID	KERATOSULFATE	CHONDROITIN Sulfate A	CHONDROITIN Sulfate C	CHONDROITIN Sulfate B	HEPARITIN Sulfate
JEANLOZ NOMENCLATURE	SAME	KERATAN-SO_4	CHONDROITIN 4-Sulfate	CHONDROITIN 6-Sulfate	DERMATAN Sulfate	HEPARAN Sulfate
Richest Tissue Sources	Vitreous humor, Skin Umbilical cord, Synovial fluid	Cornea, Cartilage, Nucleus pulposus	Aorta, bone, cartilage, cornea, Ligamentum nuchae	Cartilage, Nucleus pulposus, Tendon	Skin, aorta, heart valves, Hurler's disease urine and tissues	Aorta, lung, Hurler's urine and tissues, Amyloid
Simple Sugar components	Glucuronic Glucosamine	Galactose Glucosamine	Glucuronic Galactosamine	Glucuronic Galactosamine	Iduronic Galactosamine	Glucuronic Glucosamine
Particle size Molecular wt.	1 - 4 million (av. 1.8)	Unknown not dialyzable	50,000	50,000	Unknown not dialyzable	Variable slowly dialyzable
Type and Location of sulfate sugar-carbon number	None	Hexosamine 6-O-Sulfate	Hexosamine 4-O-Sulfate	Hexosamine 6-O-Sulfate	Hexosamine 4-O-Sulfate	$\frac{1}{2}$-N-Sulfate $\frac{1}{2}$-O-Sulfate Location Unknown
Enzymatic Digestion	Testicular Bacterial and Leech Hyaluroni-dases	Coccobacillus extract also active on blood group substances	Testicular Hyaluronidase and Adapted Proteus Vulgaris ext.	Testicular Hyaluronidase and Adapted Proteus Vulgaris ext.	Adapted Flavobacterium Extract	Heparin-adapted flavobacter-ium extract
Glycosidic Linkages Hexosamine to uronic	β(1 to 4)	N-Ac-Glucosam. Galactose β(1 to 3)	β(1 to 4)	β(1 to 4)	β(1 to 4)	Unknown
Uronic to Hexosamine	β(1 to 3)	Galactose to N-Ac-Glucosam. β(1 to 3)	β(1 to 3)	β(1 to 3)	α(1 to 3)	Unknown
Optical Rotation	-51° to -97°	+4.5°	-28° to -33°	-16° to -22°	-55° to -63°	+38° to +.78°

Collagen Metabolism (9)



Method of Determination of Urinary Hydroxyproline (10)



Hydroxyproline (From Acid Hydrolyzed Sample)

Pyrrole-2-Carboxylic Acid

(Determined by formation of a colored compound with Ehrlich's Reagent)

List of Conditions in which Urinary Hydroxyproline Peptide Excretion is Abnormal. Normal = 32 mg. ± 20 mg./24 hours on a gelatine-free diet.

Growing children (11)
Marfan's (12)
Acromegaly (13)
Dwarfism or arrested growth (13)
Gorlin-Chaudhry Syndrome (15)
High Gelatin (but not normal meat) diet (11)

CASE I: [redacted]

This 8 WF was admitted to [redacted] for a controlled metabolic study of the effect of human growth hormone. Her past medical work-up at [redacted] had established her diagnosis as one of an isolated growth hormone defect with thyroid and adrenal function studies within normal limits. PBI 6.38%, 17-Keto steroids, 1.05 mg./24 hours, normal ACTH test.

Past History: Born of a supposedly premature 7-month gestation with a birth weight of 3 lb. 5 oz., she had always been small. Some respiratory distress at birth, but afterward developed normally. No neurological symptoms, walked at 15 months, talked at 1 year. Only 4 teeth at 2 years of age. Pt. does well in school, but at age 6 had an average height of a 2 year old, and on admission at age 8 had a height of an average 2½ year old and a bone age of 3 years, 6 months.

Hospital Course: On a controlled diet the pt. showed no weight change and a remarkably constant urinary total hydroxyproline excretion of 14 mg. per day. On the 8th day of hospitalization, she was given 2½ mg. of human growth hormone/day, and this continued for 4 weeks. She showed a weight gain of 700 grams and increased her height 2 cm. during this period. Urinary total hydroxyproline increased steadily to 63 mg. per day - a normal value for a child of this age.

Follow-up: On discharge, growth hormone was stopped and 24-hour urines collected at weekly intervals. The excretion of hydroxyproline then fell steadily to 18 mg./day 3 weeks after growth hormone cessation. The pt. continued to feel quite well during and following hospitalization.

CASE II: [redacted] (Patient of Dr. James R. Hyslop)

This 43 WM [redacted] operator was admitted [redacted]-61 because of arthritis of the upper extremities and dull headaches over the forehead radiating to the base of the neck unrelieved by aspirin and increasing in intensity over a 3-year period. He was told by his friends that his hands were larger in 1948 and was convinced of this himself in 1952 and afterward when his wedding ring had to be sawed off and expanded 3 successive times.

In 1957, he began to have persistent muscle and bone aches diagnosed by his family physician as "arthritis." In 1958, he had onset of increasing muscular weakness, especially notable with exertion, and in late 1958 noticed some limitation of motion of both elbows. He then developed pain and tenderness of elbows and shoulder girdle pain and had to quit his job. In 1960 his voice became hoarse and he noted decrease in libido. He noted no change in head or jaw size, but nose is believed to have grown bigger.

He had not observed polyuria or polyphagia.

Physical Examination: A tall, muscular, intelligent WM with prominent supraorbital and malor ridges, large nose, thick lips, with a large tongue and hoarse speech. He showed normal visual fields. Forearms, wrists, hands and fingers bilaterally equally quite enlarged with squared-off digits. The joints especially seemed larger than usual, and the elbows showed limitation of motion.

Laboratory: ESR, 10; negative rheumatoid factor tests; abnormal glucose tolerance test with glucosuria; alk. phos, 4.7; phos., 2.8 mg.%; Ca, 10.7 mg.%; P.B.I., 5.4 mg.%; RaI uptake, 15.1%. Skull X-ray showed enlarged sella turcica and large frontal sinuses. Bones showed a coarsened trabecular pattern with digits showing soft tissue enlargement and prominent unguinal tufts. Elbows showed extensive degenerative arthritis bilaterally. Urinary total hydroxyproline ranged from 100-120 mg./24 hours.

Diagnosis: Acromegaly with secondary degenerative arthritis.

Course: Pt. received 3200 R tumor dose to the pituitary over a month period with minimum ill effects. His urinary hydroxyproline dropped to 90 mg./24 hours. He was then discharged and followed as an outpatient with continued improvement. Urinary hydroxyproline was again checked 3 months after irradiation and had dropped to 60 mg./24 hours.

BIBLIOGRAPHY

General Reference

1. Smiley, J.D. and Ziff, M. "The Metabolism of Connective Tissue," Ann. Rev. Med. 13 (1962) 14 pp. (In press)

Connective Tissue Metabolism

2. Hodge, A. J. and Schmitt, F. O. "Electron Microscopic Studies of Collagen," Proc. Natl. Acad. Sci. U.S. 46, 186 (1960).

3. Jackson, D. S. "Chemistry of Collagen" in Inflammation and Diseases of Connective Tissue, 32 (L. C. Mills and J. H. Moyer, Ed.; W. B. Saunders Co., Philadelphia, Pa., 1961).

4. Clark, F. and Grant, J. K., Eds. Biochemical Society Symposium No. 20, "The Mucopolysaccharides," 125 pp. (The University Press, Cambridge, 1961).

5. Morris, C. C. and Godman, G. C. "Fibroblast Synthesis of Collagen and Mucopolysaccharides," Nature 188, 407 (1960).

6. Boucek, R. J., Noble, N. L. and Woessner, J. F. "Collagen and Mucopolysaccharide Formation in Polyvinyl Sponge Implants," in Connective Tissue, Thrombosis and Atherosclerosis, 193 (I. H. Page, Ed.; Academic Press, Inc., New York, 1959).

7. Böstrom, H. and Roden, L. "Mucopolysaccharide Biosynthesis," Biochemical Pharmacology 6, 100 (1961).

8. McKusick, V. Heritable Disorders of Connective Tissue, 2nd Ed., 333 pp. (C. V. Mosby Co., St. Louis, 1960).

9. Gross, J. "Collagen," Sci. American 204, 120 (1961).

10. Prokop, D. and Udenfriend, S. "Simple Accurate Method of Hydroxyproline Determination," Anal. Biochem. 1, 228 (1960).

Urinary Hydroxyproline Peptide Excretion

11. Ziff, M., Kibrick, A., Bresner, N. and Gribetz, N. J. "Urinary Hydroxyproline Excretion," J. Clin. Invest. 35, 579 (1956).

12. Sjoerdsma, A., Davidson, J. D., Udenfriend, S. and Mitoma, C. "Urinary Hydroxyproline Excretion in Marfan's Disease," Lancet 2, 994 (1958).

13. Jasin, H., Fink, C., Smiley, J.D. and Ziff, M. "Effect of Growth on Urinary Hydroxyproline Peptide Excretion," Am.

Rheum. Assn. Meeting, December 9, 1961, Washington, D. C. (Abstract).

14. Jasin, H. and Ziff, M. "Effect of Soluble Collagen Pool on Level of Urinary Hydroxyproline Peptide Excretion in Lathyrism," Unpublished observations.

15. Gorlin, R. J. and Chaudhry, A. P. "A New Heritable Disorder of Connective Tissue," N. Eng. J. Med. 263, 1151 (1960).

Growth Hormone Effects on Connective Tissues

16. Banfield, W. G. "Effect of Growth Hormone on Soluble Collagen of Skin of Old Hamsters," Proc. Soc. Exptl. Biol. & Med. 97, 309 (1958).

17. Muir, H. "Growth Hormone Effect on Mucopolysaccharide Biosynthesis," in ref. (4), p. 4, (1961).

18. Ellis, S., Huble, J. and Simpson, M. E. "Cessation of Skin Mucopolysaccharide Biosynthesis after Hypophysectomy," Proc. Soc. Exptl. Biol. & Med. 84, 603 (1953).

19. Selye, H. and Bois, P. "On the Influence of Various Hormones upon the Development of Experimental Lathyrism," Rev. Canad. de Biol. 15, 281 (1956).

20. Selye, H. and Bois, P. "Effet des Hormones Adaptives sur le Lathyrisme Experimental du Rat," ACFAS, 24e Congrès, Montreal, 2-4 Nov. (1956).